PAPER # 11

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT	:	BEELMAN et al.
SERIAL NO	:	10/091,367
FILED	:	March 5, 2002
TITLE	:	PROCESS FOR ANTIMICROBIAL TREATMENT OF FRESH PRODUCE, PARTICULARLY MUSHROOMS
Grp./A.U.	:	1764
Examiner	:	Bhat, Nina Nmn
Conf. No.	:	7301
Docket No.	:	P07187US00

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME CITED PATENT FOR PUBLICATION (CFR 1.131)

COMMISSIONER FOR PATENTS
P. O. Box 1450
Alexandria, VA 22313-1450

Dear Assistant Commissioner:

1. This Declaration is to establish completion of the invention in this application in the United States at a date prior to May 1, 2001, the earliest effective date of the publication Journal of Food Protection, Vol. 64, No. 5, 2001 by Koseki et al. entitled "Decontamination of Lettuce Using Acidic Electrolyzed Water" that was cited by the Examiner in a §103 rejection.

2. The attached declaration of prosecuting attorney Heidi S. Nebel shows that this issue of Food Protection was mailed to customers on May 1, 2001, and is thus the earliest effective date for the publication. A journal is a prior art reference as of the date it is received by the intended recipients. *In re Schlittler*, 235 F.2d 882 (CCPA 1956).

3. The persons making this Declaration are the inventors.

4. This declaration is submitted prior to final rejection.

5. Prior to May 1, 2001, we had completed the invention described and claimed in the above identified patent application, including the concept of using acidic electrolyzed water.

6. To establish the date of completion of the invention of this application, the following attached documents are submitted as evidence:

a. The document is a grant proposal which was revised by the co-inventors to include proposing the use of acidic electrolyzed water in the initial washing step. At page 14 of the grant which shows revision marks which indicate information added in the April 26th revision, The paragraph entitled "Electrolyzed Oxidizing Water" (EO) proposes the general concept of use of EO for washing mushrooms. The attached computer record shows that this revision was made on April 26th. Prior to this date we had conducted the experiment informally at our labs at Penn State University, as a proof of principal before proposing it in the grant application, and this work is described in the paragraph.

7. The invention in this application was completed at least by that date of April 26, 2001 which is a date earlier than the effective date of the Koseki reference. These documents corroborate the same reference.

DECLARATION

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the willful false statements and the

like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURES

8A. Inventors

Full name of sole or first inventor: Robert B. Beelman

Inventor's Signature: Robert Beelman

Date: 5-24-05 Country of Citizenship USA

Residence: 710 Cornwall Rd. State College, PA 16803

Post Office Address: NA

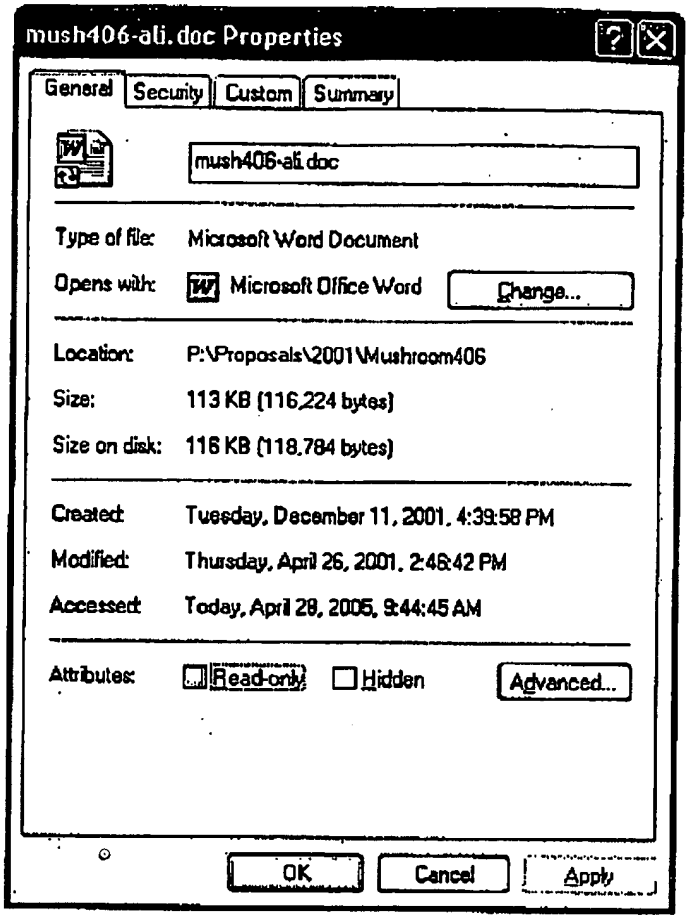
Full name of second joint inventor, if any: Ali Demirci

Inventor's Signature: Ali Demirci

Date: 5/24/05 Country of Citizenship TURKEY

Residence: 116 Thorndale Rd. Port Matilda, PA 16870

Post Office Address: NA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Opens with: Microsoft Office Word
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Size: 113 KB (116,224 bytes)
Size on disk: 116 KB (118,784 bytes)
Created: Tuesday, December 11, 2001, 4:39:58 PM
Modified: Thursday, April 26, 2001, 2:46:42 PM
Accessed: Today, April 28, 2005, 9:44:45 AM
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Heidi S Nebel

From: Robert Bruce Beelman [rbb6@psu.edu]
Sent: Thursday, April 28, 2005 9:27 AM
To: Heidi S Nebel
Subject: FW: USDA 406 Proposal

mush406-ali.doc
(119 KB)

Heidi,

This is Ali Demirci's draft of the USDA proposal sent to Luke Laborde (senior author of proposal) on April 26, 2001.

Regards,

Robert Beelman
Professor of Food Science

> ----------
> From: Ali Demirci
> Sent: Tuesday, April 26, 2005 1:30 PM
> To: Robert Bruce Beelman
> Subject: USDA 406 Proposal
>
> > <<mush406-ali.doc>>
> Bob:
>
> Here is the draft proposal from USDA 406 program in which EO water was
> included, but later on was removed due to budget cut.
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>
>
> Ali
>
>
>
> **
>
> Ali Demirci, Ph.D.
> Assistant Professor
> 231 Agricultural Engineering Building
> Department of Agricultural and Biological Engineering
> The Pennsylvania State University
> University Park, Pennsylvania 16802
> (814) 863-1098 (O)
> (814) 867-6009 (H)
> (814) 863-1031 (Fax)
> e-mail:demirci@psu.edu
> http://www.abe.psu.edu/demirci
>
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Food Safety for the Fresh Mushroom Industry: Developing a Research and Extension Education Model for the Produce Industry

I. PROJECT SUMMARY

Multidisciplinary expertise from two land grand institutions will be used to develop and implement a science-based food safety program for the fresh mushroom industry. Our overall goal is to develop a model for responding to the needs expressed by growers and packers of fresh produce for practical methods to develop effective food safety plans based on established HACCP principles and tested educational delivery methods. Specific objectives are to determine the incidence of human pathogens in manure-containing compost, casing materials, mushroom growing and packing environments, and on fresh mushrooms and to investigate survival of human pathogens under commercial composting practices. We propose to develop and validate pre- and post-harvest treatments that will ensure safe mushroom products and, at the same time, advance the field of microbial intervention technologies for fresh produce in general. Finally, we will develop and assess food safety training strategies that take into account specific attributes and preferences of primarily Hispanic harvesters. We will use our established extension education programs to deliver our findings to the mushroom industry.

II. PROJECT DESCRIPTION

This project fits within category 5: Improving the Safety of Fresh Fruits and Vegetables (Program Area 111E) because it focuses on improving the safety of fresh and minimally processed imported and domestic fruits and vegetables including the development of (a) safe and efficacious techniques to enhance or ensure microbiological safety and (b) approaches that relate to post-production, harvesting, handling, transportation, and distribution control measures to prevent microbial pathogen infection or cross-contamination.

A) INTRODUCTION

- **Need for an educational and research model to address food safety issues for the fresh produce industry**

In April of 1999, representatives from government, academia, and growers' groups met in Orlando Florida to attend the *National Educational Conference: Toward Implementing the "Guide to Minimizing Microbial Food Safety Hazards in Fresh Fruits and Vegetables"*. The conference was intended to identify the education needs of domestic growers and producers of fresh fruits and vegetables and ways to best implement the FDA "Guide". Among the conclusions drawn during the 2 days of discussion was the need to develop a nationwide model for educating growers about food safety issues related to fresh fruits and vegetables while at the same time considering the unique practices, and therefore, potential hazards associated with specific commodities. In her concluding comments to the participants Dr. Jan Singleton (National Program Leader, Food Science and Food Safety, USDA, CSREES) stated, "....we

don't want to develop an education model or an outreach program from ground zero if there already is something out there that is successful. If anything, we want to tap into it.".

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) recommended that Good Agricultural Practices (GAP) for use on the farm and Good Manufacturing Practices for use in packinghouses be developed (De Roever, 1999). The committee additionally concluded that while Hazard Analysis and Critical Control Point Programs (HACCP) will afford the best assurance of safe produce, there is not enough data at the present time to institute such programs for fresh produce.

In addition to collecting data for use by the mushroom industry, this proposal seeks to develop a nationwide model for responding to the needs expressed by growers and packers of fresh produce for practical methods to develop effective food safety plans based on established HACCP principles and tested educational delivery methods. We will focus our efforts on the mushroom industry because we have already identified and achieved a record of cooperation and support from stakeholder groups including national mushroom growing organizations, individual companies, and buyers of fresh mushrooms. In addition, there is a need to evaluate specific and unique hazards associated with growing, harvesting, and packing of fresh mushrooms and to develop technological and educational methods to prevent their occurrence.

Two land grant universities from the leading mushroom growing states, Pennsylvania and California have already formed collaborations in extension education for the mushroom industry and now propose to extend the relationship to research activities. The resources and multi-disciplinary expertise within Penn State University and the University of California-Davis, their close proximity to growers and packers, and a history of successful collaboration with the mushroom industry will increase the probability that the results of the proposed independent research will be transformed into workable food safety programs.

- **Consumer Preference for Fresh Produce and Increased Foodborne Illness in the United States**

Within the last two decades, consumption of fruits and vegetable in the United States has steadily risen as consumers perceive fresh or minimally processed products to be a healthful alternative to more processed foods. Per capita consumption (farm weight) of fresh fruits and vegetables increased by 26% between 1970 and 1997 from 254 to 319 lbs. In recent years, the fresh-cut produce industry has rapidly expanding with sales projected to rise from $8 billion in 1999 to $19 billion by 2003 (Greenleaf, 1999). The U.S. government and major health associations, including the National Research Council and National Cancer Institute have contributed to this rise by strongly recommending consumption of at least 5 servings of fruits and vegetables per day to maintain adequate intake of vitamins, anti-oxidants, and essential trace elements (National Research Council, 1989; Kennedy et. al, 1996).

At the same time, there has been an increase in the incidence of reported cases of foodborne illness. The Centers for Disease Control estimates that, in the United States, there are approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths per year due to consumption of food contaminated with pathogenic microorganisms (Mead et al., 1999). Three pathogens, *Salmonella* spp., *Listeria monocytogenes*, and *Toxoplasma gondii*, are responsible for 1,500 deaths each year, more than 75% of those caused by known pathogens, while unknown agents account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3,200 deaths. Particularly susceptible to foodborne illness are the very young, the elderly, and other individuals

with impaired immune systems. The CDC has further estimated that the number of reported cases associated with consumption of fruits and vegetables has increased five-fold between the periods 1988-1992 and 1993-1997 (Bean, et al., 1996; Olsen, 2000). Of particular significance have been outbreaks attributed to contamination of tomatoes and cantaloupes with *Salmonella* spp., imported raspberries with Cyclospora parasites, Mexican strawberries with Hepatitis A virus, alfalfa sprouts with *Salmonella* spp. and *E. coli* O157:H7 bacteria, and unpasteurized juices with *Salmonella* spp., *E. coli* O157:H7, and *Cryptosporidium* (Beuchat, 1998).

Changes in agronomic, harvesting, distribution, processing, and consumption patterns have contributed to this increase (Beuchat and Ryu, 1997). Human pathogens such as *Listeria monocytogenes, Clostridium botulinum,* and *Bacillus cereus* are naturally present in soils, and their presence on fresh produce is not unexpected. *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, parasites, and viruses may contaminate fresh produce through the use of raw or inadequately composted manure, irrigation water containing untreated sewage, or contaminated wash water. Harvesters may also become a source for contamination if proper personal hygiene practices are not followed. Improperly cleaned and sanitized equipment surfaces in growing, packing, and handling environments are known reservoirs for pathogenic *Listeria* and thus are likely vehicles for contamination of fresh produce (Suslow, 2000). Contamination of fresh fruits and vegetables is thought to be potentially more serious than contamination of animal products because these foods are more likely to be consumed without having been subjected to treatments that reduce levels of pathogenic microorganisms (Doores, 1999). The number of reported outbreaks and product recalls will no doubt increase as consumption of fresh and minimally processed foods increases, methods for detection of human pathogens improve, better diagnostic and tracking tools are developed, and the proportion of susceptible individuals in the general population increases.

- **Response by government and industry to foodborne disease outbreaks**

As a result of these outbreaks, international and domestic standards for safe growing and packing of fresh produce have been developed by a number of governing and advisory agencies. U.S. standards for safe growing and packing of fresh produce were issued by the Food and Drug Administration recently in the document, "Guide to Minimizing Microbial Food Safety Hazards on Fresh Fruits and Vegetables" (FDA, 1998) and a "Code of Compliance" has been developed for the produce industry in Europe (CIEH, 1999). International farm-to-fork food safety standards for the food industry have been established by the World Health Organization's (WHO) "Codex Alimentarius - Recommended International Code Of Practice General Principles Of Food Hygiene" (WHO, 1997). In addition, legislation before the United States Senate proposes mandatory conformance to good agricultural and manufacturing practices that would, in effect, force growers and packers to comply with sanitation regulations and inspection procedures similar to that which food processors already face (Federal Register, 1999).

Although these documents currently provide only voluntary guidelines and have no regulatory authority, produce distributors, major retail grocery, foodservice chains, and other wholesale buyers of fresh fruits and vegetables are increasingly relying on these standards as the basis for mandating sanitation specifications and food safety plans for growers and packers. In 1999, Safeway Inc., with over 1600 grocery stores throughout North America, initiated a program requiring third-party food safety audits of its suppliers of "high-risk" produce (Linden, 1999). Initially limited to leaf lettuce, the retailer has plans to expand the program to other high-

risk items and ultimately to all fruits and vegetables it purchases. Shortly thereafter, Albertson's Inc., with over 2000 stores nationwide, asked its suppliers of all fresh produce items to verify safe production and packing practices. Specifically required were the development of safe production manuals and regular self- and third-party audits based on the sanitation standards provided in the FDA's "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables". A&P, Supervalu, and Kroger, the nations largest retail food chain, as well as Burger King, Subway, Wendy's and other fast food chains have also favored third-party audits as a means of assuring conformance to good agricultural and manufacturing practices. A number of industry organizations have interpreted these standards and issued guidelines to packers, and fresh-cut processors (IFPA, 2000; PMA, 1998; PMA, 1999; AIB, 1999).

As government, industry, and consumer scrutiny has intensified, extension specialists have increasingly been called upon to assist small and medium sized growers and packers of fresh fruits and vegetables develop food safety plans that satisfy these demands (LaBorde, 2000)

- **Economic significance and distribution of the mushroom industry**

Although considered a minor crop, commercial mushroom production makes a significant contribution to the total Unites States agricultural output. Sales of all mushrooms in 1999-2000 reached a record high of 867 million pounds valued at $867 million (USDA, 2000). The common white mushroom (*Agaricus bisporus*) accounts for 82% of total domestic sales. Mushroom cultivation is regionally concentrated in two states. In Pennsylvania, 86 growers account for approximately 52% of domestic mushrooms sold. Twenty-one growers in California, the next largest producer, contribute an additional 15 percent of the domestic crop. Growers in the U.S. tend to be small to medium size family owned operations with approximately 2/3 producing less than 5 millions lbs/year.

Mushroom consumption trends reflect the overall consumer preference for fresh and minimally processed fruits and vegetables. Within the last 10 years, mushroom production has increased by 31%. In 1999-2000, U.S. fresh market production was 78 percent of total sales volume with processed mushrooms making up the remaining 22 percent. This is in sharp contrast to 1970-1971 figures where only 32% of the total volume was for the fresh market and 72% were processed. In California, 90% of the crop is sold as fresh product.

Changes in the demographics of mushroom harvesters mirrors that of the produce industry as a whole (Flammini, 1999). The industry, which was started by Pennsylvania Quakers in the 19th century, grew rapidly in the 1920s when Italian immigrants performed the work. A succession of poor immigrants followed and in the 1950s, growers began recruiting poor southern whites and African-Americans followed by Puerto Ricans until well into the 1970s. Since then, poorer Mexican workers gradually replaced the Puerto Rican workforce in the late 1970s until today where approximately 98% of the workforce is composed of Mexican laborers. An effective food safety educational program must, therefore, take into account the specific attributes and preferences of Hispanic harvesters.

- **Current mushroom growing practices**

Commercial mushroom growing is a unique form of agriculture in that it requires decomposed organic matter as a growth substrate and source of essential nutrients (Schisler, 1982). A traditional substrate mixture contains horse and/or chicken manure, hay, corn cobs,

straw, brewers grain, cotton seed and cocoa seed hulls and is aerobically fermented under carefully controlled conditions in a process known as Phase I composting (Anon., 2001a). Phase I substrate preparation is usually performed outside or in covered structures. Selected ingredients are mixed and formed into long rows that are periodically turned, watered, and reformed. Complex microbiological and chemical changes occur as nutrients are converted into forms that can be more efficiently utilized for subsequent mushroom. Rapid microbial growth causes the compost temperatures to reach as high as 175°F (80°C). Completion of Phase I occurs after 15 to 25 days when the substrate becomes pliable, dark-colored, and has a distinct ammonia odor. Although the temperatures achieved in the Phase I process are theoretically sufficient to kill human pathogens, temperature distribution within the rows is uneven and, therefore, may permit survival and growth of pathogens on the periphery of the rows.

Phase II begins when the completed Phase I substrate is transferred to enclosed growing houses where further composting activity and nutrient conversion occur. There are regional differences in how the compost is transferred to the mushroom houses. In the Eastern United States, compost is typically added to permanent beds within the mushroom houses while, in the West, the compost is more likely to be added to specialized trays that are then transferred to the mushroom houses. Phase II includes a controlled pasteurization step that is designed to eliminate mushroom pathogens, weeds, and insect pests. A successful crop requires that the substrate temperature reach approximately 60°C for at least 2 hours (Wuest, 1982). These conditions may be achieved by heat naturally evolved during continued fermentation or by addition of steam into the mushroom growing house. Guidelines for Phase II time/temperature treatments have historically been developed to achieve optimal yields and have not taken into consideration effects on human pathogens.

Mushroom spawn, consisting of laboratory-inoculated sterile cereal grains, is then worked into the substrate and a casing layer, usually a peat moss/lime mixture, is applied. Growth of mycelium occurs throughout the substrate and into the casing layer where, after two to three weeks, mushroom fruiting bodies are formed. Mature mushrooms are harvested, trimmed, and packed into boxes for further processing or machine-slicing or are packed directly into plastic tills for retail sale. Because moisture accelerates microbial growth and reduces shelf-life (Guthrie, 1984; Guthrie and Beelman, 1989), most growers do not wash their product before packing thus allowing small amounts of adhering casing material on the mushroom surface (Beelman, personal communication, April 20, 2001).

- **Potential for contamination of mushrooms with human pathogens**

In any fruit and vegetable packing facility, there is a potential for cross contamination from soil, humans, animals, agricultural irrigation sources, decaying plant residue on equipment or bins, cull piles, packing sheds and fresh-cut processing systems (Suslow, 2000). In the case of mushroom operations, storage of animal manures and composting operations occur within close proximity to growing, packing, and post-harvest handling operations and thus pose a serious potential for cross contamination. The possibility that significant numbers of human pathogens might survive the composting and pasteurization processes should, therefore, be assessed and systematic, preventative procedures developed.

Horse and poultry manures used as compost ingredients are likely sources of hazardous bacteria such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, and perhaps *E. coli* O157:H7 (Heuvelink, et al., 1999; Himathongkmam and Riemann, 1999; Himathongkmam

et. al, 2000). There are, however, no published studies on the incidence of human pathogens in mushroom compost and casing ingredients or the effect of Phase I and Phase II composting on pathogen survival. Preliminary studies conducted at the Penn State Mushroom Test Demonstration Facility (MTDF) indicate the potential for survival of harmful bacteria. Coliform bacteria were enumerated from samples collected from poultry and horse manures, Phase I rows (at the center of the pile and at the periphery), and after Phase II pasteurization at 140°F for 2 hours (Table 1). As expected, coliform bacteria were numerous in the raw horse manure/straw mixture. Low levels in dried poultry manure can be attributed to the use of a commercially prepared oven-dried poultry manure product at the MTDF. Most mushroom farms use untreated poultry manure that undoubtedly contains greater amounts of bacteria. Despite Phase I temperatures of up to 180°F within the interior of the row and repeated mixing, high levels of bacteria survived at the periphery of the rows. The Phase II steam pasteurization treatment of 140°F for 2 hours, however, reduced coliforms to undetectable levels. These results demonstrate that bacteria are capable surviving the Phase I composting process and that controlled and monitored pasteurization can significantly reduce their numbers. However, commercial practices vary widely and there are no data to provide guidance to growers on the minimum lethal heat treatments required to reduce human pathogens to safe levels while at the same time maintaining an adequate crop yield.

Table 1. Estimation of coliform bacteria in mushroom compost

Compost ingredient / process step	Coliforms (CFU/g)
Dried poultry manure	nd*
Raw horse manure / straw mix	7.7×10^6
Phase I (periphery of row)	4.3×10^7
Phase I (center of row)	3.8×10^3
Phase II pasteurized (140°F/2 hr)	nd*

*none detected (<10 CFU/g)

There have been no reported outbreaks associated with consumption of fresh mushrooms. However, there have been several reports of human pathogens isolated from fresh mushrooms. Doyle and Schoeni (1990) isolated *Campylobacter jejuni* in 3 of 200 (1.5% frequency) retail, polyvinyl chloride film-wrapped packages of fresh mushrooms purchased at several Midwest retail outlets. The authors speculated that contamination might have occurred through contact with inadequately pasteurized compost, casing material, or by hand contact. A survey of retail mushrooms in the Pacific Northwest conducted in 1999 revealed that 1 and 5% of samples were positive for *Listeria monocytogenes* and *Salmonella* spp., respectively (Samapour et al., 1999). In a recent incident in Ireland (Anon., 2001b), samples of fresh mushrooms tested positive for *Salmonella* kedougou prompting authorities to advise consumers to thoroughly cook all mushrooms before eating.

Because of the intensive hand labor required to harvest, trim, and pack fresh mushrooms, the potential for contamination with *Staphylococcus aureus* in commercial operations must be considered. Doores et al. (1986), using fresh mushrooms grown at the Penn State Mushroom Test Demonstration Facility, confirmed the presence of *Staphylococcus*. Moreover, several outbreaks of staphylococcal food poisoning associated with canned mushrooms produced in China have been attributed to contamination during picking with growth and toxin formation occurring during long distance, un-refrigerated transport (Beelman, 1990).

Mushrooms actively respire during storage and can rapidly deplete oxygen levels in unventilated packages. Hardt-English and coworkers found that headspace oxygen levels in unventilated bags of mushrooms dropped from atmospheric levels (20%) to 2-3% within 4-6 hours at room temperature. Under these conditions, facultative anaerobic bacteria, including the human pathogens mentioned above, can have a competitive advantage over aerobic spoilage microorganisms and thus grow rapidly to dangerous levels. Studies at Penn State have demonstrated that rapid growth of *Staphylococcus aureus* and formation of enterotoxin occurs in unventilated packages of fresh inoculated mushrooms held at 20-30°C (Martin and Beelman, 1996). Evidence that *Clostridium botulinum* spores can germinate and form deadly toxin in unventilated packages of mushrooms held at room temperature prompted the Food and Drug Administration to advise the mushroom industry to properly ventilate packages using perforated PVC film (Kautter, et al., 1978).

The proximity of mushrooms to manure-containing compost, the amount of hand contact required to harvest them, and their high respiration rate suggests that contamination and microbial growth does occur in fresh mushrooms. A high level of process control is required to prevent inadequate film perforation, inadvertent label placement over ventilation holes, and temperature abuse to prevent depletion of oxygen and subsequent pathogen growth within mushroom packages.

Given, the growing trend for consumption of fresh and minimally processed mushroom products that are not subjected to microbial reduction treatments, educational programs and microbial intervention technologies are needed to reduce these hazards to acceptable levels.

- **Technologies for Disinfection of Fresh Fruits and Vegetables**

Washing Methods. A wide variety of antimicrobial agents have been used on fresh produce (Beuchat, 1998). However, development of effective washing treatments for mushrooms has proved to be a difficult challenge for researchers. McConnell (1991) conducted a review of potential wash additives for mushrooms including sodium hypochlorite, hydrogen peroxide, potassium sorbate, and sodium salts of benzoate, EDTA, acid pyrophosphate, hexa-metaphosphate, and pyrophosphate. In each case, shelf life was reduced because the wash treatments resulted in absorption of water and accelerated growth of spoilage microorganisms that caused rapid browning to occur (Guthrie, 1984; Guthrie and Beelman, 1989). Although there are no published studies on wash treatments for reduction of pathogens on mushrooms, new technologies deserve to be investigated.

High pH washing. Previous research at Penn State demonstrated that Gram-negative pathogens, such as *E. coli* O157:H7 and *Salmonella* spp., are rapidly destroyed by high pH wash treatments (Catalano and Knabel, 1994; Mendonca, et al, 1994; Woody, 2000). Exposure to a high pH (at or above pH 11) and temperatures (at or above 100°F) interacts synergistically to kill Gram-negative pathogens by disrupting membranes. Beelman and Duncan (1998) developed a multiple stage wash procedure for mushrooms, with an initial high-pH anti-microbial step, followed by one or more pH neutralization/browning inhibitor washes. The process was highly effective in physically removing debris as well as delaying microbial spoilage. However, the authors did not determine the efficacy of the process in destroying human pathogens.

Ozonated water. Ozone has been proven to be a more effective antimicrobial than the most commonly used disinfectant, chlorine, against a wide range of microorganisms. It has been used safely in water treatment plants for nine decades, especially in Europe and the US. The Food and Drug Administration approved ozone as generally recognized as safe (GRAS) for disinfection of bottled water (FDA, 1995) opening new doors for other applications including its use for decontamination of minimally processed fruits and vegetables.

Electrolyzed oxidizing water. Electrolyzed oxidizing (EO) water is a newly recognized disinfecting solution that has the potential to be used for inactivation of pathogenic microorganisms associated with produce surfaces. The generation of EO water involves reactions in a cell containing inert, positively charged and negatively charged electrodes, separated by a membrane, and through which a very dilute salt water solution passes. By subjecting the electrodes to direct current voltage, two types of water possessing different characteristics are generated: an electrolyzed basic aqueous solution containing dilute sodium hydroxide (NaOH) produced at the cathode and an electrolyzed acidic solution containing dilute hydrochloric acid (HCl) produced at the anode. The antimicrobial activity of EO water appears to be due to the achieved oxidation-reduction potential as well as the presence of hypochlorous acid (Kim et al., 2000). Recent tests in have demonstrated that *Escherichia coli* O157:H7 and *Listeria monocytogenes* when exposed to EO water for two minutes were reduced by 7 $\log_{10}$ cfu/ml or 99.99999%. Reductions of >5 $\log_{10}$ cfu/100 cm^2 of *E. coli* O157:H7 and *L. monocytogenes* were observed when cutting boards were treated with EO water for 10 minutes. While these studies clearly illustrate that EO water is an effective antimicrobial for reducing foodborne pathogens in water and on cutting boards, no studies have been conducted to determine efficacy of EO water in decontaminating mushrooms.

Irradiation.

Details needed Bob Beelman - In the United States, irradiation of up to 10kGy for decontamination of spices has been used commercially since the early 1980's and that the FDA approved the use of 1 kGy on fruits and vegetables for the purpose of insect and/or growth and maturation control in 1986. In 1964 the FDA approved the use of 0.05 to 0.15 kGy for potatoes for inhibition of sprouting.

Survival of microorganisms during irradiation depends on several functions (Farkas, 1989). These are the number, nature, and the inherent ability of the cell to withstand these assaults and undergo repair of microorganisms. Resistance also depends on extracellular environmental conditions such as pH, temperature, and chemical composition of the food. Numerous studies have been done to determine resistance or the measurement of D values (decimal reduction, or dose required to destroy 90% of the microorganisms present). Gram-negative microorganisms were found to be more sensitive than Gram-positive microorganisms. Gram-negative bacterial pathogens including *E. coli, Yersinia enterocolitica, Aeromonas hydrophila,* and *Campylobacter,* have a relatively low resistance to irradiation (El-Zawahry and Rowly, 1979; Lambert and Maxcy, 1984; Palumbo, et al., 1986), and they can be destroyed at a dose of 1.5 kGy. However, a dose 1.5-3.0 kGy is needed to destroy about 99.9% of *Salmonella,* because *Salmonella* among Gram-negative pathogens may be the most resistant except spores of *Clostridium botulinum.*

- **Ongoing research, extension, and education programs related to mushroom production and processing**

Research. Penn State University has a long and productive relationship with the mushroom industry and works closely with the two national commodity groups; The American Mushroom Institute in Washington D.C. and the American Mushroom Council in Dublin, California. The Penn State Department of Plant Pathology is home to the Mushroom Research Center (MRC) and the Mushroom Test Development Facility (MTDF), an interdepartmental, production-orlented facility for conducting research related to engineering, crop management, pest and disease control, and economics of commercial mushroom production.

In addition, faculty members from the Department of Food Science are conducting research on new cultural practices and/or minimal processing technologies to improve product quality while maintaining safety and controlling spoilage. The mushroom industry has traditionally supported research projects that provide information on practices that increase productivity and quality attributes. There is, however, a definite and immediate need for independent research on pre- and post-harvest practices that ensure safe mushroom products.

Extension. Penn State Cooperative Extension has maintained strong ties with the mushroom industry. Extension specialists from several departments within the College of Agricultural Sciences conduct problem-solving research and work with county agents in major mushroom growing areas to deliver information to growers and packers. Each year for the past 43 years, Penn State hosts a mushroom industry short course that has averaged over 300 participants from 20-25 states and several foreign countries. This venue provides excellent opportunities to present research findings to the industry on a timely basis.

Food safety education for mushroom growers and packers is ongoing in the Department of Food Science. As part of his Cooperative Extension appointment, Dr. LaBorde has utilized linkages with the mushroom industry to study methods for delivering food safety programs to the fresh and minimally processed fruit and vegetable industry. He has conducted two workshops on food safety issues and HACCP plan development for the mushroom industry. The first workshop, held on November 16-17, 1999 in Avondale, Pennsylvania, was attended by 64 people from 37 mushroom companies and related industries from Pennsylvania, Connecticut, New Jersey, Tennessee, Washington, and Oregon. A second workshop in San Jose, California, was attended by 33 individuals from California and Oregon and included Dr. Linda Harris of U.C.- Davis as a guest lecturer.

Education. There are many opportunities for students to learn about mushroom technology and microbial food safety at Penn State. The College of Agricultural Sciences offers a Mushroom Science and Technology Minor that includes courses in mycology, the biology and management of mushroom pests, and independent study courses that emphasize practical experience at the Mushroom Research Center and Mushroom Test Demonstration Facility. The Department of Food Science offers undergraduate and graduate education courses in basic and applied food microbiology, detection and control of foodborne pathogens, and microbial diversity and offers both undergraduate and graduate students numerous opportunities for conducting research related to microbial food safety. In addition, the undergraduate lecture and laboratory course "Science and Technology of Plant foods" uses mushrooms in 4 of 15 laboratory sessions to teach students concepts in fruit and vegetable quality improvement, minimal processing technologies, and microbial food safety intervention techniques.

B) OBJECTIVES

Although efforts are focused on the mushroom industry, our overall objective for this proposal is to develop a model for responding to the needs expressed by growers and packers of fresh produce for practical methods to develop effective food safety plans based on established HACCP principles and tested educational delivery methods.

Specific objectives in this proposal are as follows:

1) **Determine the incidence of human pathogens in (a) mushroom compost ingredients and casing materials, (b) commercial mushroom growing, packing, and fresh-cut processing environments, and (c) fresh mushrooms delivered for retail sale.**

2) **Determine the survival of human pathogens during Phase I and Phase II commercial composting procedures and conduct validation experiments to support Phase II pasteurization as a Critical Control Point.**

3) **Develop and validate pre- and post-harvest microbial intervention technologies for disinfection of mushrooms.**

4) **Develop and assess food safety training strategies that take into account specific cultural attributes of Hispanic workers.**

5) **Provide the mushroom industry with practical procedures and training materials for developing and implementing food safety programs in their operations.**

C) METHODS

Objective 1. Determine the incidence of human pathogens in (a) mushroom compost ingredients and casing materials, (b) commercial mushroom growing, packing, and fresh-cut processing environments, and (c) fresh mushrooms delivered for retail sale.

Details required – L. Harris, T. Suslow, B. Beelman

(a) Animal manures used as compost ingredients are potential sources of contamination of nearby packing and fresh-cut processing areas. It is, therefore, necessary to determine the types of human pathogens present in compost ingredient and casing materials and to determine the extent of cross contamination that occurs in commercial mushroom packing operations.

Survival of human pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *E. coli* O157:H7) during composting will be determined by surveying several commercial mushroom farms in the Northeastern and Western regions of the US. Operations that use a traditional horse and chicken manure-based compost (Schisler, 1982) will be selected to minimize ingredient variation. Samples of raw ingredients, phase I compost, phase II pasteurized compost, and casing materials will be collected and transported to the Department of

Food Science at Penn State and the University of California–Davis for microbial analysis as proscribed by the Compendium of Methods for the Microbiological Examination of Foods.

Samples of poultry manure and horse manure/straw mixtures used as compost ingredients will be collected at several commercial mushroom farms in Pennsylvania and California. Samples will be evaluated for total APC, coliforms, generic *E. coli*, and the amounts of *Salmonella*, spp. *Campylobacter*, *E. coli* O157:H7, and *Listeria* present using methods described by the Compendium of Methods for the Microbiological Examination of Foods or the FDA Bacteriological Analytical Manual.

Anecdotal reports indicate that that some samples of peat used in mushroom casing formulations may also contain soil-borne human pathogens. Because the casing layer is applied after Phase II pasteurization, it has the potential to contaminate mushrooms. Casing material often adheres to the surface of mushrooms and, because most mushroom growers and packers do not wash their product before packaging (Beelman, personal communication, April 20, 2001), it is important to determine the incidence of human pathogens such as *Salmonella*, *Campylobacter*, *E.coli* O157:H7, and *Listeria* in casing materials and their potential for growth.

(b) Equipment and facilities in growing, packing, and fresh-cut processing areas in cooperating facilities will be sampled to determine the presence of human pathogens. Total APC, coliforms, generic *E. coli*, *Salmonella*, *Campylobacter*, *E. coli* O157:H7, and *Listeria* will be enumerated using methods described by the Compendium of Methods for the Microbiological Examination of Foods or the FDA Bacteriological Analytical Manual.

(c) Mushrooms packed in 8 oz. ventilated, packages will be obtained from several packing facilities in the Eastern and Western US within one day of harvest. Samples will be evaluated for total APC, coliforms, generic *E. coli*, and the amount of *Salmonella*, *Campylobacter*, *E.coli* O157:H7, and *Listeria* present.

Objective 2. Determine the survival of human pathogens in mushroom compost during Phase I and Phase II commercial composting operations and conduct validation experiments to support Phase II pasteurization as a Critical Control Point (CCP).

Details required

The effect of Phase II pasteurization parameters on pathogen survival will be determined by using traditional horse and chicken manure-based compost that has completed the phase I process. The compost will then be non-thermally pasteurized using UV or gamma irradiation to eliminate background microflora while avoiding heat-induced chemical and physical changes to the matrix. The compost will be inoculated with the most heat resistant pathogens to give final inoculum levels of 10^5 CFU/g. Inoculated compost will then be subjected to temperatures between 54 and 66°C for up to 4 hours and number of surviving pathogens will be determined.

Objective 3. Develop and validate pre- and post-harvest microbial intervention technologies for disinfection of mushrooms.

Pre-harvest mushroom cultivation, inoculation, analysis methods – Bob Beelman

Post-harvest inoculation, analysis methods – Bob Beelman, Ali Demirci, Linda Harris

High pH washing.

Details required – B. Beelman

Experiments will follow the procedures developed by Beelman and Duncan (1998) using a multiple stage wash procedure with an initial high-pH anti-microbial step followed by one or more pH neutralization/browning inhibitor washes.

Ozone.

~~**Details required – T. Suslow, A. Demirci**~~

Efficacy of ozonated water for decontaminating mushrooms will be determined. Mushrooms will be inoculated by dipping in cell suspensions of *E. coli* O157:H7, *L. monocytogenes*, or *Salmonella* spp containing 10 to 1000 cfu/ml of the pathogen. Ozone generated by a lab scale ozone generator (Hess Machine International, Ephrata, PA) will be purged trough an inlet line and stainless steel sparger (10 µm pore size) in sterile water at a specific flow rate and for a specific time until the desired ozone concentration is achieved. At the exit line, excess ozone will be passed through 1% potassium iodine solution to prevent the ozone from being released into the environment. Twenty-five grams of inoculated samples will be immersed in 1 liter of ozonated water according to the following parameters: *I)* Ozone concentration (2-10 mg/L), *II)* Contact time (1-15 minutes), and *III)* Effect of agitation during treatment. The temperature of ozonated water will be maintained at 4°C. Sufficient ozone availability and reaction time are two main factors for successful inactivation of pathogens. Ozone concentration and agitation will play important roles in amount of available ozone. A proper reaction time is needed to achieve desirable microbial inactivation. Therefore, these test parameters need to be optimized for inactivation of pathogenic microorganisms. After each treatment degree of inactivation will be determined by appropriate microbiological analysis. If this method proves to be effective, sensory analysis will be performed on the treated samples to ensure quality of the final product.

Electrolyzed oxidizing water.

The efficacy of electrolyzed oxidizing (EO) water for decontaminating mushrooms will be determined. The samples will be inoculated by dipping in cell suspensions of *E. coli* O157:H7, *L. monocytogenes* or *Salmonella* spp at inoculum levels of 10 to 1000 cfu/ml. EO water will be prepared with a ROX20TA EO water generator (Hoshizaki Electric Company Ltd, Japan). A 12% solution of sodium chloride and deionized water will be pumped simultaneously into the equipment. After achieving a steady-state condition, the EO water will be collected into pre-sterilized containers. The contaminated samples will be treated by submerging in EO water for various time periods, and the population of surviving pathogens will be determined. For this process, the following parameters

will be optimized: *i)* Temperature of EO water, *ii)* Contact time, *iii)* Mixing by either stirring or circulating EO water.

Objective 4. Develop and assess food safety training strategies that take into account specific cultural attributes of Hispanic workers.

Objective 5. Provide the mushroom industry with practical procedures and training materials for developing and implementing food safety programs in their operations.

D) PARTICIPATING INSTITUTIONS, DEPARTMENTS, AND PERSONNEL

- Pennsylvania State University - Lead Institution
  - Department of Food Science
    Luke F. LaBorde, Ph.D., Assistant Professor and Project Director, 70% Extension/30% Research
    Robert. B. Beelman, Ph.D., Professor 70% Research/30% Teaching
    J. Lynn Browne, Ph.D., Associate Professor, 70% Extension/30% Research
  - Department of Plant Pathology
    David M. Beyer, Ph.D., Assistant Professor, 70% Extension/30% Research
  - Department of Agricultural and Biological Engineering
    Ali Demirci, Ph.D., Assistant Professor, ~~70~~55% Research/~~30~~45% Teaching
- University of California-Davis
  - Department of Food Science and Technology
    Linda J. Harris, Ph.D., Associate Professor
    Maria de la Fuente, PhD., University of California Cooperative Extension

The following industry groups support the goals of the research and extension programs in this proposal:

The American Mushroom Institute, One Massachusetts Avenue, N.W., Washington, D.C. 2001

The American Mushroom Council, 11875 Dublin Blvd. Suite D 262, Dublin, CA 94568

E) EQUIPMENT AND FACILITIES

Penn State Department University

The Department of Plant Pathology's Mushroom Research Center (MRC) provides unique facilities for conducting mushroom research. The MRC contains a spawning-casing area, two phase-2 rooms, two spawn-production rooms, eleven production rooms, and an analytical-clinical lab. The Mushroom Test Development Facility (MTDF) is an interdepartmental, production-oriented facility designed to integrate into one system the most

desirable developments of engineering, crop management, pest and disease control, and economics research on the commercial mushroom. The MTDF is equipped with the normal equipment found in a commercial growing facility as well as computerized environmental controls. The MTDF supplies fresh mushrooms daily supporting researchers from Plant Pathology, Ag and Biological Engineering, Entomology, and Food Science, as well as visiting faculty from other universities. The Gammacell 220 High Dose Rate Research Irradiator (Nordion International Inc., Ontario, Canada) available at the Penn State Breazeale Nuclear Reactor uses a ^{60}Co gamma irradiation source which can deliver up to 2.7 kGy/hr. Ozone generator and electrozed oxidizing water generator are readily available in Department of Agricultural and Biological Engineering.

General equipment available in the Department of Food Science pertinent to this proposal include: clinical, micro, high-speed, and ultra centrifuges, French press, ultrasonic cell disrupter, liquid scintillation counter, speed-vac concentrator, lyophilizer, particle analyzer, pH meters, fluoroptic temperature probes, data acquisition system, high performance liquid, gas, and thin layer chromatography, atomic absorption, infrared, UV-VIS-NIR spectrophotometer, ELISA plate reader, miniVIDAS™, electrophoresis units, phase, light, fluorescent microscopes equipped with at television monitor, incubators spanning all temperature ranges, humidities, carbon dioxide and aeration levels, and an anaerobic glove box and incubator.

University of California-Davis

Need details – T. Suslow and L.Harris

F) PROJECT TIMETABLE

III) REFERENCES

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IV) VITAS OF PI'S

V) CONFLICT-OF-INTEREST LIST

VI) LETTERS OF SUPPORT/COLLABORATIVE ARRANGEMENTS

VII) COLLABORATIVE AND/OR SUBCONTRACTUAL ARRANGEMENTS

VIII) BUDGET NARRATIVE

IV) CURRENT AND PENDING SUPPORT (FORM CSREES-663)

X) ASSURANCE STATEMENT(S), (FORM CSREES-662)